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RAPD-PCR BASED STUDY OF JUMPING SPIDERS FROM AGRICULTURAL FIELDS OF AMRAVATI DISTRICT, MAHARASHTRA (INDIA)

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ABSTRACT

The current study deals with the genetic diversity of jumping spiders using molecular markers. A total 831 scorable bands were produced using six random primers for the 23 species of jumping spiders belonging to saltisidae family. Out of all screened primers, OPN 16 produced 265 scorable bands and OPP 9 produced 164 polymorphic bands. Remaining primers (OPA 2, OPA 3, OPA 4 and OPA 5) showed 100 per cent polymorphism with 139, 84, 84 and 85 bands respectively. With few exceptions the phylogenetic relationship of jumping spiders using UPGMA and NJ approach was in agreement with the classical systematics. The present study is the first report from India that describes the genetic relatedness amongst spider using RAPD-PCR.

Key words: genetic diversity, jumping spiders, RAPD-PCR, molecular markers,

INTRODUCTION

Spiders are group of invertebrate belonging to class Arachnida and occur almost in every habitat. Arachnids constitute the second largest class (7%) of documented arthropods and it is estimated that (8.3%) of arthropods are arachnids. Order Araneae includes 110 families of 3849 genera and 42473 species, among these family salticidae (jumping spiders) contains 574 genera and 5368 species throughout the world (Platnick, 2011). In India, 1520 species belonging to 377 genera and 60 families has been reported. Recent status of salticidae contains 66 genera and 192 species (Sebastian, 2009). Salticids spiders are active, hunting spider capable of jumping or leaping to a distance. Jumping spiders are diurnal,move by walking, running, jumping or leaping and uses all these movements for prey capture. They hunt prey by stalking, chasing and leaping over it. Salticidae are capable of recognizing colors and distinguishing the prey from considerable distance. Most characteristic feature is the ocular quadrangle on the cephalothoraxes deliminited by eight eyes arranged in three or four rows, anterior median eyes are very large and easily noticeable that's why it is called as jumping spider.

At the starting of nineteen century, Pocock (1900) studied on several species of spiders in his book 'fauna of British India'. Firstly, after 1960, Tikader (1967,

1973b) worked out several new species of jumping spiders. Sadana (1991) described salticids spiders from north India. Biswas (1984a, b) gave account of few new species belonging to the family salticidae. As like, Bastawade (2002) also contributed to jumping spiders. Biswas (1998b, 2004) reported new species of salticids spiders from Madhya Pradesh. Side by side Sebastian (2009) published his work on the South Indian spider.

Hedin (2001) investigated molecular phylogeny and evolution of jumping spiders. Maddison and Hedin (2003) sequenced several jumping spiders to understand evolutionary relationship with other families of spiders. Arnedo (2001) studied the monophyletic relation among the species of salticids spider. Maddison (2008) studied evolutionary relationship amongst the salticids spider.

Virtually, no work has been attempted from Indian region to infer evolutionary status of jumping spider by using DNA based molecular markers .The current research is about study of genetic relatedness among jumping spider using RAPD-PCR.

To summarize, taking into account the absolute unavailability of study on Salticid spiders using DNA marker, the study was undertaken to provide insight into their genetic diversity and systematic from agricultural fields of Amravati district.

MATERIALS AND METHODS

Study area: Amravati district is located in the state of Maharashtra-India. It is at $20^0$55' and 20.93 North latitude $77^0$45' and 77.75 East longitudes. It has an average elevation of 343 meters (1125 feet). Amravati has tropical wet and dry climate with hot, dry summer from April to June. The annual average rainfall in the district is 852.1 mm and temperature recorded between 18^0C to 46^0C (Falling rain Genomics, inc. 2010).

Collection, preservation and identification: Species of jumping spiders (Salticidae) were collected by using sweep netting, beating sheets, active searching and hand picking methods from the Agricultural fields of Amravati district. Adult male and female were identified under stereo zoom microscope with the existing keys by Tikader (1980), Gajbe (2004) and Sebastian (2009). Collected spider species were preserved in 70% ethanol.

DNA extraction, PCR and electrophoresis: The DNA was extracted from fresh spider legs using DNASure Tissue mini kit (Genetix Biotech) by method as supplied with the kit. RAPD-PCR was performed in 30ìl reaction using 1.5ìl (7.5 units) of Taq DNA polymerase (Fermentas, USA), 1.5ìl Taq buffer 10x (fermentas, USA), Dream Taq MasterMix 12.5ìl, 3ìl primer (10mM, Operon technology) and 1ìl (50

to 100ng) diluted genomic DNA. The remaining volume was made with 10.5ìl nuclease free water in 0.2ìl PCR tube. The Master cycler Gradient (eppendrof) was used to perform the Polymerase Chain Reaction (PCR). Cycling condition was as follows.
Pre-denaturation: 95^0C for 3 min,
Denaturation: 95^0C for 1 min,
Annealing: 36^0C for 1 min,
Extension: 72^0C for 1 min,
Final extension: 72^0C for 8 min, 42 Cycle.
10ìl PCR amplification products along with 2 ìl of DNA loading dye were separated on 2 per cent Agarose gel containing Ethidium bromide (0.5 ìl 1/10 ml of gel) at 110 volts using 1x TBE buffer. The gel were viewed under Kodak Gel Logic 212 Imaging System and photographed for further analysis. Out of 20 primers only six (Operon technology, USA) primers (10 bp) were used for RAPD-PCR **(Table. 1).**

Data analysis: The entire six random primers scored data was combine into single binary matrix to perform cluster analysis by the program 'Mesquite' *(Maddison and Maddison; 2007). Existing file saved in the form of 'NEXUS', which was used as input file for* 'PAUP' (Swofford; 2003) for the construction of phylogenetic tree i. e. UPGMA and Neighbor joining were mainly adopted for clustering methods.

RESULTS

A total 831 scorable bands were produced using six random primers for the 23 species of jumping spiders. Out of all primers, OPN 16 produced highest i. e. 265 scorable bands with 100% polymorphism (Figure 1). OPP 9 produced 164 polymorphic and one monomorphic band (Figure. 2). The percent of polymorphism was 99.27. Remaining primers OPA 2, OPA 3, OPA 4 and OPA 5 produced 139, 84, 84 and 85 bands with 100 per cent polymorphism respectively. Finally, six primers in 23 species of jumping spiders produced 831 bands, of which 830 were polymorphic thereby exhibiting 99.87 per cent of polymorphism. From the above data, each primer produced 138.5 bands of which 138.33 bands were polymorphic. The per cent of polymorphism was 99.87 per primer (Table. 2).

Genetic distance: Genetic distance refers to the genetic divergence between species or between populations within a species. Smaller genetic distance indicates a close genetic relationship whereas large genetic distance indicates distant genetic relationship. Based on RAPD data genetic distance of 23 jumping spider were calculated using MEGA-5 evolutionary software (Tamura *et al.* 2011). Mainly, two methods Neighbor-Joining and UPGMA were considered for phylogenetic tree construction (Table. 3).

The genetic distance was computed from the pooled data to construct the phylogenetic tree. The RAPD-PCR based distance matrix shows, maximum genetic distance i.e (0.417) between the species *plexippus paykulli* and *Marpissa species1*.

Table No. 1: Six random primers were used for RAPD-PCR profiling of Salticidae spiders

Primer	Sequence5' to 3'	% of GC content
OPA 2	TGCCGAGCTG	70%
OPA 3	AGTCAGCCAC	60%
OPA 4	AATCGGGCTG	70%
OPP 5	AGGGGTCTTG	60%
OPP 9	GTGGTCCGCA	70%
OPN 16	AAGCGACCTG	60%

Table No. 2: Scorable DNA bands generated by six random operon primers through RAPD-PCR.

Sr. No.	Primers	Total no. of band produced	No.of Polymorphic bands	Percent of Polymorphism
1	OPA 2	139	139	100
2	OPA 3	84	84	100
3	OPA 4	84	84	100
4	OPA 5	95	95	100
5	OPP 9	164	163	99.27
6	OPN16	265	265	100
Total		831	830	
Pooled		138.5	138.33	99.87

Table No. 3: Genetic distance matrix of jumping spider computed using MEGA-5.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	21	22	23
01		0																				
02	0.347	0																				
03	0.417	0.306	0																			
04	0.403	0.306	0.25	0																		
05	0.319	0.306	0.389	0.306	0																	
06	0.368	0.313	0.34	0.34	0.215	0																
07	0.41	0.368	0.354	0.285	0.271	0.264	0															
08	0.403	0.292	0.292	0.292	0.292	0.243	0.285	0														
09	0.375	0.333	0.278	0.333	0.25	0.201	0.326	0.25	0													
10	0.389	0.361	0.361	0.361	0.306	0.285	0.326	0.292	0.236	0												
11	0.368	0.299	0.326	0.326	0.285	0.292	0.306	0.271	0.257	0.354	0											
12	0.382	0.34	0.354	0.368	0.313	0.292	0.347	0.313	0.271	0.34	0.292	0										
13	0.299	0.271	0.299	0.299	0.188	0.181	0.208	0.257	0.16	0.243	0.25	0.306	0									
14	0.347	0.361	0.319	0.361	0.278	0.229	0.285	0.306	0.264	0.333	0.299	0.299	0.243	0								
15	0.396	0.271	0.299	0.243	0.299	0.278	0.264	0.271	0.285	0.299	0.292	0.347	0.222	0.299	0							
16	0.375	0.347	0.375	0.347	0.25	0.313	0.326	0.361	0.278	0.319	0.368	0.368	0.201	0.319	0.285	0						
17	0.313	0.313	0.313	0.326	0.257	0.25	0.306	0.271	0.188	0.285	0.25	0.264	0.153	0.243	0.25	0.201	0					
18	0.389	0.319	0.347	0.347	0.292	0.271	0.368	0.333	0.208	0.306	0.313	0.257	0.201	0.319	0.313	0.264	0.215	0				
19	0.326	0.313	0.34	0.326	0.215	0.25	0.278	0.285	0.243	0.285	0.25	0.278	0.181	0.299	0.278	0.243	0.194	0.243	0			
21	0.326	0.313	0.368	0.313	0.313	0.278	0.319	0.34	0.299	0.34	0.375	0.292	0.25	0.299	0.319	0.313	0.236	0.257	0.264	0		
22	0.375	0.306	0.319	0.361	0.292	0.257	0.326	0.264	0.222	0.236	0.34	0.313	0.215	0.264	0.271	0.264	0.229	0.25	0.285	0.257	0	
23	0.34	0.299	0.354	0.368	0.271	0.319	0.403	0.368	0.299	0.285	0.403	0.319	0.25	0.368	0.375	0.326	0.306	0.285	0.264	0.278	0.299	0
23	0.25	0.212	0.354	0.368	0.271	0.319	0.312	0.368	0.299	0.285	0.403	0.345	0.25	0.368	0.375	0.326	0.306	0.285	0.256	0.278	0.199	0.234

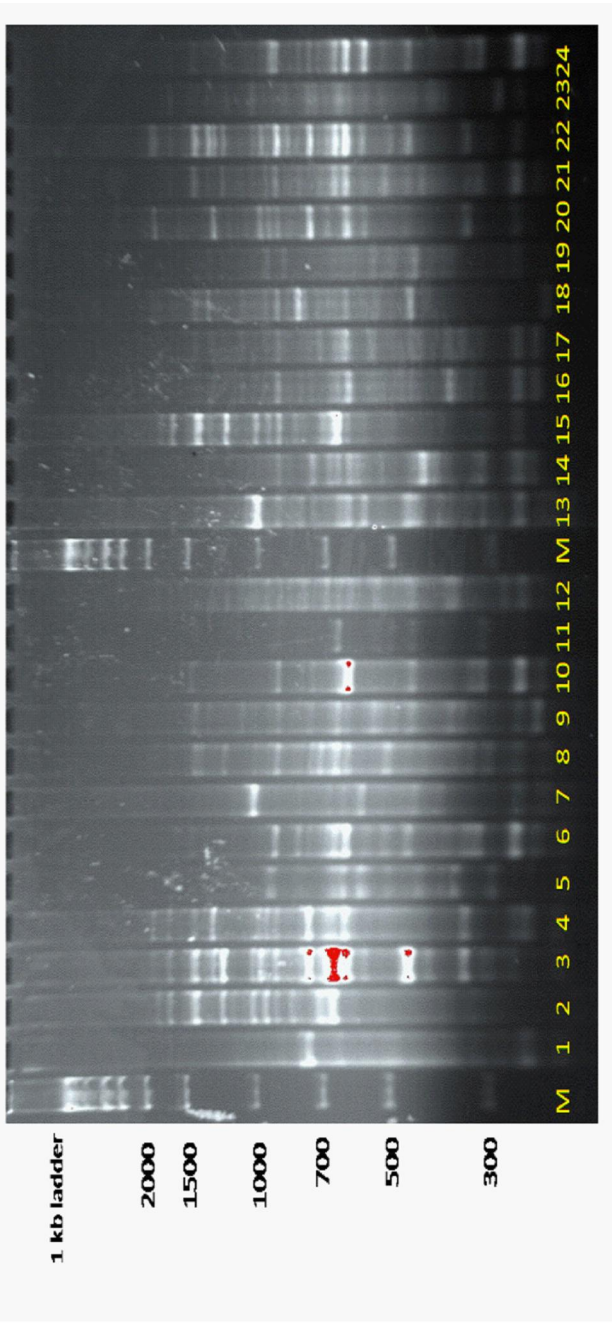

Figure No. 1: RAPD-PCR screening by OPN 16 primer of family Salticidae.

M- marker, 1- *Plexipus paykulli*, 2- *Thiania species1*, 3- *Marpissa species1*, 4- *Marpissa bengalensis*, 5- *Myrmarachne bengalensis*, 6- *Phintella vittata*, 7- *Hyllus semicupreus*, 8- *Marpissa species2*, 9- *Phidippus species1*, 10- *Harmochirus brachitus*, 11- *Telomonia elegans*, 12- *Phidippus species2*, 13- *Ptocasius species1*, 14- *Plexipus pertersi*, 15- *Hyllus species1*, 16- *Bavia species1*, 17- *Brettus species1*, 18- *Phintella versicolor*, 19- *Telomonia dimidiata*, 21- *Hasarius adansoni*, 22- Unknown species, 23- *Menemerus species1*. 24-*Phidippus species3*.

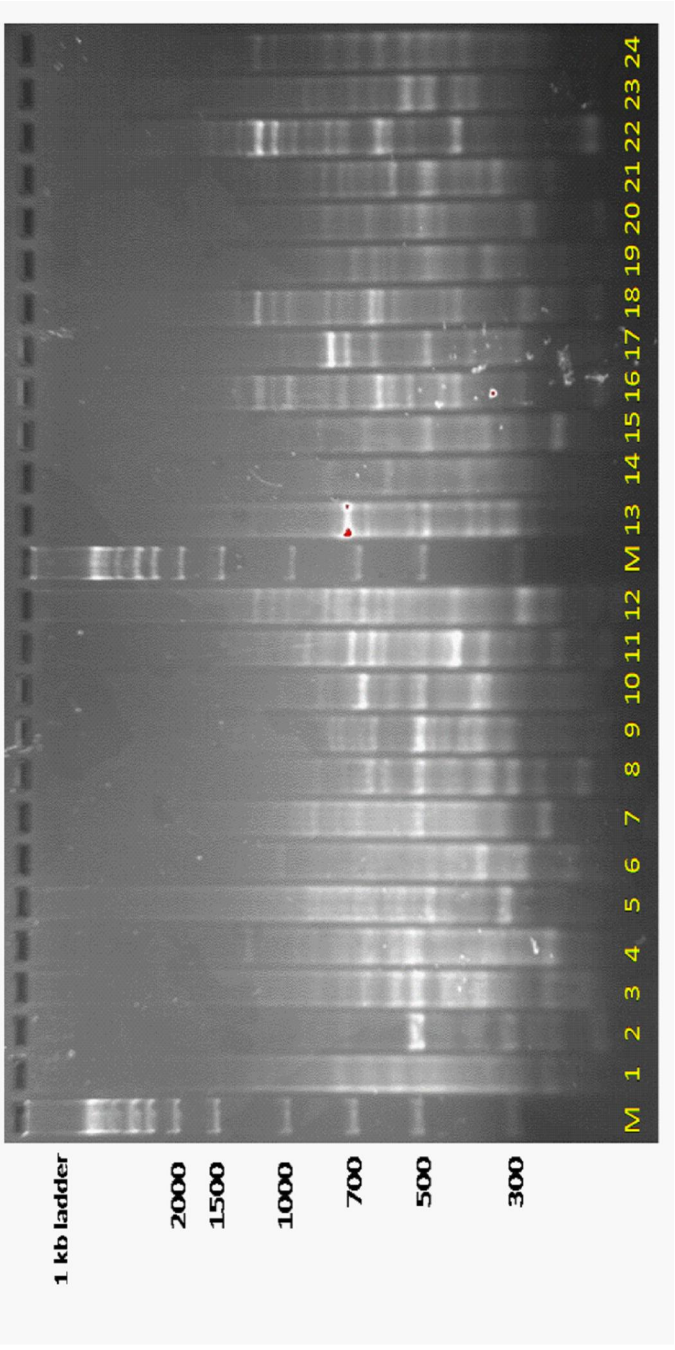

Figure No. 2: RAPD-PCR screening by OPP 9 primer of family Salticidae.

M- marker, 1- *Plexipus paykulli*, 2- *Thiania species1*, 3- *Marpissa species1*, 4- *Marpissa bengalensis*, 5- *Myrmarachne bengalensis*, 6- *Phintella vittata*, 7- *Hyllus semicupreus*, 8- *Marpissa species2*, 9- *Phidippus species1*, 10- *Harmochirus brachitus*, 11- *Telomonia elegans*, 12- *Phidippus species2*, 13- *Ptocasius species1*, 14- *Plexipus pertersi*, 15- *Hyllus species1*, 16- *Bavia species1*, 17- *Brettus species1*, 18- *Phintella versicolor*, 19- *Telomonia dimidiata*, 21- *Hasarius adansoni*, 22- Unknown species, 23- *Menemerus species1*. 24-*Phidippus species3*.

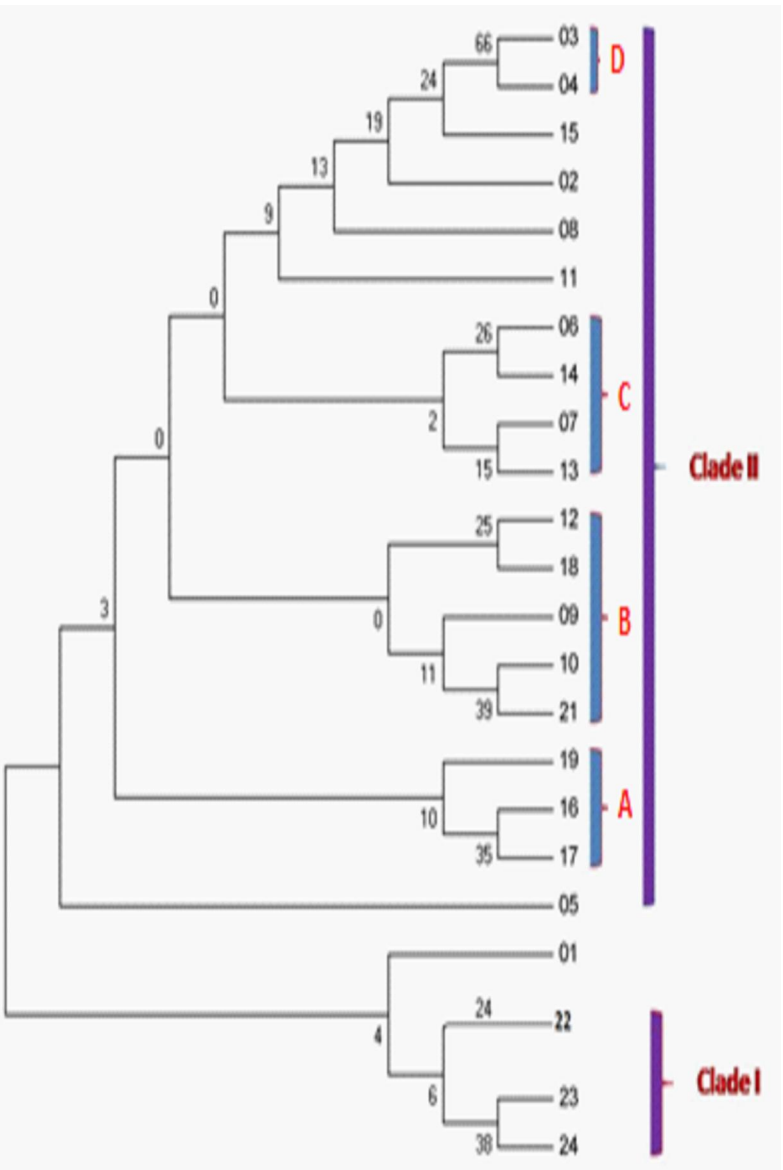

Figure No. 3: UPGMA tree of jumping spiders.

M- marker, 1- Plexipus paykulli, 2- Thiania species1, 3- Marpissa species1, 4- Marpissa bengalensis, 5- Myrmarachne bengalensis, 6- Phintella vittata, 7- Hyllus semicupreus, 8- Marpissa species2, 9- Phidippus species1, 10- Harmochirus brachitus, 11- Telomonia elegans, 12- Phidippus species2, 13- Ptocasius species1, 14- Plexipus pertersi, 15- Hyllus species1, 16- Bavia species1, 17- Brettus species1, 18- Phintella versicolor, 19- Telomonia dimidiata, 21- Hasarius adansoni, 22- Unknown species, 23- Menemerus species1. 24- Phidippus species3

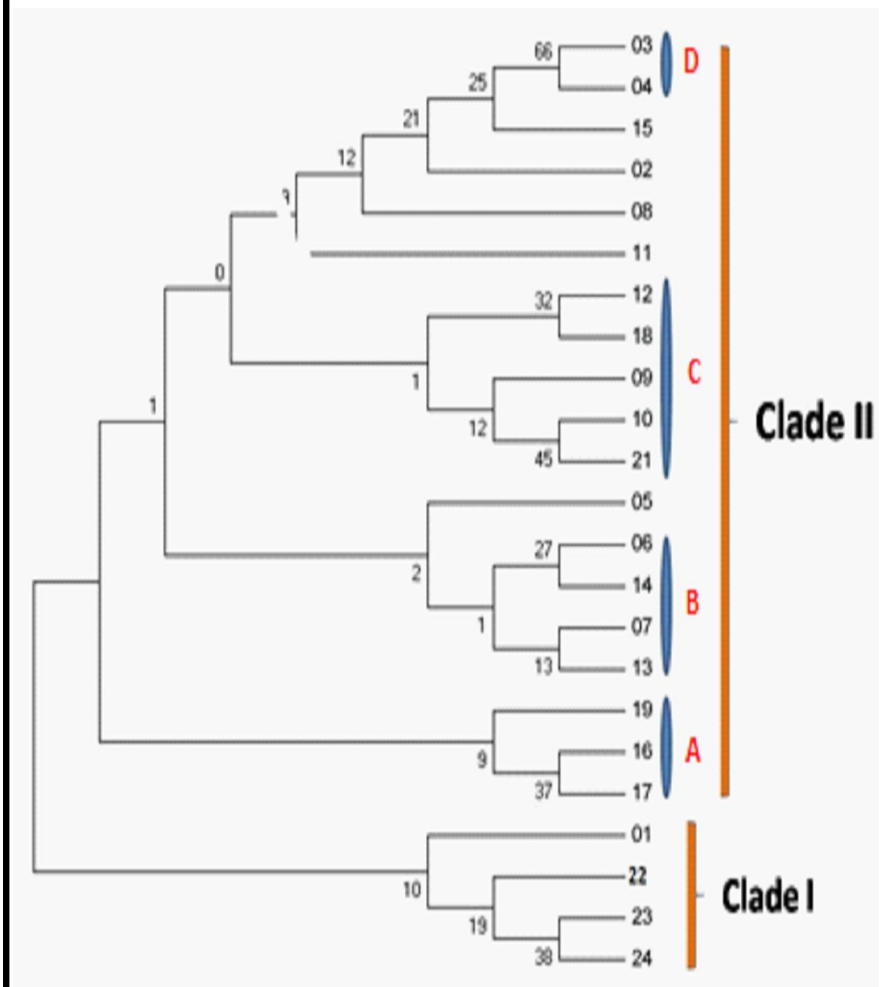

Figure No. 4: Neighbor joining tree of jumping spiders.

Minimum genetic distance (0.34) was found between the species *Plexippus paykulli* and *Menemerus species1*.

Evolutionary relationship of jumping spiders

UPGMA tree: Phylogenetic tree construction of jumping spiders using UPGMA approach show splitting of taxa into two major clades, designated as clade-I and clade-II. Clade-I contains an unknown species which grouped with the *Menemerus species1* and *Phidippus species3* and forms a monophyletic clade. On the other hand, clade-II comprises of four sub-clade IIA to IID. Clade-IIA exhibit monophyly of *Telomonia dimidiata, Bavia species1* and *Brettus species1*. Clade-IIB includes *Hasarius adansoni, Harmochirus brachitus* and *Phidippus species1* while *Phidippus species1* outgroup this sister cluster. Additionally, *Phidippus species2* and *Phintella versicolor* cluster together as sister taxa. The monophyletic Clade-IIC shows clustering of *Phintella vittata* and *Plexipus petersi* as sister taxa while *Hyllus semicupreus* and *Ptocasius species1* cluster together as sister taxa. Clade-IID exhibits clustering of *Marpissa species1* and *Marpissa bengalensis* with high bootstrap value. Remaining species such as *Hyllus species1, Thiania species1, Marpissa species2, Telomonia elegans, Maramarachne bengalensis* and *Plexipus paykulli* exhibits paraphyly (Figure 3).

Neighbor-joining: Phylogenetic tree construction of jumping spiders using NJ approach also shows splitting of taxa into two major clades, designated as clade-I and clade-II. Clade-I includes an unknown species which grouped with the *Menemerus species1* and *Phidippus species3* as sister taxons while *Plexipus paykulli* outgroup this monophyletic clade. On the other hand, clade-II contains four sub-clades, viz. IIA to IID. Clade-IIA shows monophyletic relationship of *Telomonia dimidiata, Bavia species1* and *Brettus species1*. Clade-IIB contains *Ptocasius species1* and *Hyllus semicupreus*, as sister taxa while *Phintella vittata and Plexipus petersi* forms another sister cluster to which *Myramarachne bengalensis* behave as out-group.The monophyletic Clade-IIC includes *Hasarius adansoni, Harmochirus brachitus* and *Phidippus species1* as sister taxa while *phintella versicolor* and *Phidippus species2* cluster together as sister taxa. Clade-IID comprises of *Marpissa species1* and *Marpissa bengalensis* supported by high bootstrap value. Remaining species such as *Hyllus species1, Thiania species1, Marpissa species2, Telomonia elegans, Maramarachne bengalensis* and *Plexipus paykulli* exhibits paraphyly (Figure 4).

DISCUSSION

The most significant and distinguishing characteristic of salticid spiders lies in their development of vision, with their large, tubular principal eyes also known as anterior median eyes (AME). Recent work on the evolution of salticid's has been based upon the comparative study of gene sequences (Hedin and Maddison, 2001; Maddison and Hedin, 2003a, 2003b; Maddison and Needham, 2006; Su, *et al*. 2007; Maddison, *et al*. 2007, 2008; Maddison, 2009). The exceptional development of eyes in salticid spiders has supported their evolution of an

extraordinarily diverse range of lifestyles. A large number of salticid spiders are closely resembled to ants, beetles and mantis-like species.

The salticid's are a member of the RTA (Retrolateral Tibial Apophysis of males) clade, which includes 17 families of spiders. Few exceptions families are those members which lost the ability to build webs and web-building families include dictynids, agelenids, and amaurobiids (Blacklege *et al.* 2009). The origin of salticidae itself within the RTA clade must be due to the evolution of tubular and telescopic eyes (Williams and McIntyre, 1980; Hill, 2007).

Salticidae are presently grouped with other similar families into the Dionycha, those having two-clawed hunting spiders (Coddington, 2005). Evolutionary study of crab spider by Benjamin *et al*. (2008) placed them philodromidae into a basal position relative to the salticidae and form sister group of families like Corinnidae, Miturgidae, Gnaphosidae, Anyphaenidae, and Thomisidae. This study suggested that some philodromids were actually more closely related to the latter families including the salticidae. Studies on jumping spiders by Maddison and Hedin (2003a) and Maddison and Needham (2006) were consistent with the grouping of Gnaphosidae, Miturgidae, and Thomisidae into a sister clade of Salticidae. Maddison and Hedin (2003a) included several salticids into the clade *salticoida* which contain majority of all salticid species.

The present study has also pointed towards the monophyly of Salticidae. UPGMA and NJ tree mainly split into two major clade I and II (Figure 3 & 4). Clade I contain *Phidippus species3, Menemerus* as sister taxa while an unknown species exhibit early divergence. The entire clade I diverge out separately from the Clade-II during the course of divergence from a common ancestor. The Clade-II is formed by the second divergence event during which the genus *Myrmarachne* diverge from the rest of the member quite early. The occurrence of further monophyletic sub-cluster within the major clade-II, cast light on the need of revised systematic as the clade do not stick to the strict monophyletic status of the sub families discussed by earlier worker like Madison (2003, 2006, 2008).

On the other hand, major clade II further divided into four sub-clade from IIA to IID. The systematic position of all genera is not well established. In the DNA sequence phylogeny inferred by Hedin and Maddison (2003) *Brettus* behave as outlier while in this study, *Brettus* form a clade with *Bavia* and *Telomonia dimidiata* and behave as out-group.

Molecular data of *Hasarius adansoni, Harmochirus brachiatus, Phidippus species1* and their sister taxa *Phintella vorsicolor, Phidipuss species2* clearly indicates that clade IIB is monophyletic. This finding of ours was in congruent with the finding of Maddison and Hedin (2003).

Clade IIC shows two sister taxa among which *Plexippus petersi* belongs to sub-family plexippininae, *Phintella vittata* belong to sub-family Heliophaninae and falls in one sister taxa and another sister taxa which contain an exceptional

genus *Ptocasius species1* is still unclassified subfamily status with *Hyllus semicupreus* indicates the close relation with sub-family Plexippininae.

Further, Clade IID exhibit two taxa, namely, *Marpissa bengalensis* and *Marpissa species1* as sister taxa. *Hyllus species1, Thiania species1, Marpissa species2* and *Telomonia elegans* belongs to sub-family Marpissinae and dendryphantinae, and formed paraphyletic group. However their relationship was found to be uncertain and exhibit paraphyly.

The systematic of jumping spiders inferred in the current paper using RAPD based markers revealed that to obtain a highly resolve phylogeny of abundant salticid spiders, there is need of more extensive sampling spanning large geographical area followed by sequencing of multiple coding and non-coding genes.

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